PB85-218907

Current Research of the Environmental Protection Agency on Automotive Criteria Pollutants

(U.S.) Health Effects Research Lab.
Research Triangle Park, NC

1985

U.S. Department of Commerce
National Technical Information Service
NTIS

PB85-216907

EPA/600/D-85/108
1985

Current Research of the Environmental Protection Agency on Automotive Criteria Pollutants.

Judith A. Graham, Donald H. Horstman,
Vernon A. Benignus, and Robert S. Dyer
Health Effects Research Laboratory
U.S. Environmental Protection Agency

Judith A. Graham, Ph.D., is Chief of the Toxicology Branch, Inhalation Toxicology Division (ITD), Health Effects Research Laboratory (HERL). Donald H. Horstman, Ph.D, is Chief of the Physiology Section, Clinical Studies Branch, ITD, HERL. Vernon A. Benignus, Ph.D., is a Research Psychologist in the Neurophysiology Branch, Neurotoxicology Division (NTD), HERL. Robert S. Dyer, Ph.D., is Chief of the Neurophysiology Branch, NTD, HERL.

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.
EPA/600/D-85/108		PB85 218907/AS

4. TITLE AND SUBTITLE	5. REPORT DATE
Current Research of the Environmental Protection Agency on Automotive Criteria Pollutants	1985
	6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
J.A. Graham, D.H. Horstman, V.A. Benignus, and R.S. Dyer	

9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
EPA, HERL RTP, NC 27711	A9GA1A
	11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS	13. TYPE OF REPORT AND PERIOD COVERED
Office of Research & Development Health Effects Research Laboratory USEPA Research Triangle Park, NC 27711	14. SPONSORING AGENCY CODE EPA-600/11

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The HERL conducts a variety of investigations of the health effects of air pollutants to serve as criteria for the reevaluation of National Ambient Air Quality Standards. Human clinical studies include research on the effects of: 1) ozone (O_3) and nitrogen dioxide (NO_2) on exercising healthy adults, asthmatics, males vs. females, and blacks vs. whites; 2) O_3 and NO_2 on pulmonary cell biology and immunology; 3) carbon monoxide (CO) on cardiovascular physiology of patients with ischemic heart disease; and 4) CO on neurophysiology. Human research also includes investigation of the mechanisms of the effects of O_3 and NO_2 and the relationship between rate of exposure to CO and the level of carboxyhemoglobin formation and effects. The neurophysiological effects of lead are being investigated in humans and monkeys. Animal studies with O_3 and NO_2 include: 1) evaluation of the progression of chronic lung disease during chronic exposure, 2) determination of the effects on antiviral lung defenses, and 3) investigation of the mechanisms of effects. Quantitative animal-to-man extrapolation models for O_3 and NO_2 focused on dosimetry and species sensitivity, are under development.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to Public	Unclassified	16
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Introduction

The Health Effects Research Laboratory (HERL) has the primary responsibility within EPA for conducting research relative to hazard assessment of ambient air pollutants. The purpose of this research is to provide criteria for reevaluating National Ambient Air Quality Standards. To this end, epidemiological, human clinical, and animal studies are conducted. Recent studies relative to automotive criteria pollutants will be described.

Human Studies

The laboratories and exposure chambers of the Clinical Research Branch and the Neurophysiology Branch are located on the Medical School campus of the University of North Carolina in Chapel Hill. Investigators from the Clinical Research Branch and Neurotoxicology Division have developed strong collegial ties with scientists from the Medical School, especially through the UNC Center for Environmental Medicine. Working relationships have also been established with scientists from the operations, maintenance and modifications contractor, Environmental Monitoring Services. The research program described herein represents the collaborative efforts of scientists and engineers from the three groups, with EPA investigators occupying the leadership role for most of the studies.

The Clinical Research Branch consists of three sections: Physiology, Immunology and Biochemistry, and Biomedical Engineering. Each section utilizes the methodologies of their respective disciplines to address potential adverse health responses to air pollutants in human test subjects. The research objective of the Clinical Research Branch is the study of acute, reversible changes in physiological, immunological and biochemical functions resulting from controlled, short-termed exposures to low concentrations of air pollutants. Acute, reversible changes in function represent significant health end points. Recently, the Branch has expanded its research objectives to include the study of mechanisms responsible for or associated with changes in function (e.g., site and mode of action, class and extent of injury, neural pathways). In addition to establishing new autonomous health end points (e.g., lung epithelial permeability, lung tissue inflamation), results from these studies may provide the conceptual basis for relating acute, reversible changes in function with the more permanent changes in structure and function that result from chronic exposure to air pollutants.

Ozone

The study of the effects of exposure to ozone is the largest, most comprehensive clinical research program. Besides establishing additional objectives and health endpoints, the scope of subjects studied, which was initially confined to healthy, young males, has been expanded to include the study of additional subject populations, some healthy and some with preexisting disease.

Studies of Healthy Populations. Young males performing intermittent heavy exercise (minute ventilation, $\dot{V}E$ = 65 l/min) during 2-hr exposures to O_3 concentrations ranging between 0.0 and 0.4 ppm exhibit concentration dependent pulmonary function decrements and symptoms.[1] Small, but

significant decrements in function and symptoms were evident for exposure to 0.12 ppm O_3; the magnitude of decrements increased with increasing O_3 concentration. Large intersubject differences in response to any single O_3 exposure were observed. Substantial intersubject variability in response was also observed for a larger group of young males (n = 67) exposed to 0.18 ppm O_3 with heavy intermittent exercise.[2] The average decrease in 1-sec forced expiratory volume (FEV1) was 10%; 67% of the subjects had decrements less than 10%, while 33%, 15% and 9% of the subjects had decrements greater than 10%, 20% and 30%, respectively. To ascertain whether some individuals are reproducibly more responsive to O_3 than others, the magnitudes of decrements in function were compared for O_3 exposures repeated in the young males at intervals up to 14 months.[3] Decrements in forced vital capacity (FVC) and FEV1 were highly reproducible for all tested O_3 concentrations of 0.18 ppm or greater. This indicates that the magnitude of response to a single O_3 exposure is a valid estimate of an individual's intrinsic responsiveness to ozone which, in turn, explains the previously observed intersubject differences in O_3 induced responses.

The magnitude of decrement in function observed for exposure with heavy exercise suggested that decrements might also result from exposure to low levels of O_3 with exercise requiring less ventilation. To test this hypothesis, young males performed 1 hr of continuous moderate exercise (VE=40 l/min) while randomly exposed at weekly intervals to clean air or 0.18 ppm O_3. When compared to clean air exposure, small, but significant lung function decrements (FVC, 3.5% and FEV1, 6.1%) were observed following exposure to O_3. Individual FEV1 responses following exposure to O_3 ranged from +4% to -21%. Both activity and pollutant levels used in this study are typical of the real world.

The pulmonary function response of boys (ages 8-11 years) exposed once to clean air and once to 0.12 ppm O_3 in random order was determined. Exposures were for two hours and included heavy intermittent exercise. When compared to clean air, a significant decline in FEV1 was found after exposure to O_3. The magnitude of this decline was consistent with that demonstrated under similar exposure conditions in adults. A comprehensive study comparing the O_3 concentration-pulmonary function response characteristics of black versus white and female versus male subject populations is ongoing. Exposures are for 2 hr at O_3 concentrations ranging between 0.0 and 0.4 ppm with intermittent moderate exercise. Exposure of more than 300 individual subjects will be necessary to complete this study. A study evaluating the effects of 0.18 ppm O_3 on exercise performance and related physiological and perceptual variables in trained endurance runners was initiated recently. Trained runners routinely perform exercise with associated minute ventilations of greater than 100 l/min for periods in excess of 1 hr. Both of these factors result in substantial increases in effective O_3 concentrations encountered at any ambient O_3 concentration. Runners perform runs to exhaustion once in clean air and once in O_3 at exercise intensities corresponding to 80 to 95% maximal capacity. In addition to run time, measurements of arterial oxygenation, lung function, lung mechanics, lung gas exchange and perceptions of overall, respiratory and muscular exertion and pain are obtained.

To better understand the neural pathways involved in pulmonary responses to O_3, responsive subjects inhaled either saline (placebo) or atropine (a cholinergic receptor blocker) prior to 2 hr exposures to clean air or 0.4 ppm

O_3 with heavy intermittent exercise.[4] As expected, O_3-induced significant changes in lung function and symptoms. Atropine prevented the O_3-induced increase in airway resistance and partially blocked the decrease in FEV1, but did not modulate the major effects of O_3, i.e., a significant fall in FVC, a change in respiratory rate and tidal volume during exercise or the frequency and severity of respiratory symptoms. These results suggest that increased airway resistance resulting from O_3 exposure is mediated by a parasympathetic neural pathway and that changes in other variables are mediated by mechanisms not dependent upon muscarinic cholinergic receptors of the parasympathetic nervous system. Thus, at least two relatively independent mechanisms are responsible for O_3-induced changes in lung function and symptoms.

A decrease in FVC is the primary physiological consequence of O_3 exposure. Possible mechanisms of FVC reduction include decreased lung compliance (i.e., stiff lungs) and a reduction in maximal inspiratory effort mediated by vagal afferent nerve fibers probably connected to irritant receptors located in the large airways. Presently, two studies are being conducted to elucidate the role of these two mechanisms. Subjects are exposed to 0.4 or 0.5 ppm O_3 for 2 hr with heavy intermittent exercise to assure decreases in lung volume. In one study, changes in pressure-volume relationships in the lungs are evaluated after O_3 exposure to ascertain the role of compliance changes. In the other, aerosolized lidocaine, a topical anesthetic which should induce vagal afferent blockade, is inhaled after O_3 exposure and its effect on changes in FVC are determined. In addition, the correlation between responsiveness to O_3 and inhaled citric acid, as well as the effect of O_3 exposure on citric acid responsiveness, will be determined to further examine the role irritant receptors play in O_3-induced decrements in FVC. Citric acid stimulates cough receptors, and lidocaine modulates this stimulation.

Ozone-induced changes in epithelial barrier function in the airways, as indicated by changes in permeability, bioelectric properties, and biostructure also are being examined. For example, subjects are exposed to clean air or 0.4 ppm O_3 for 2 hr with heavy intermittent exercise; a wide range of changes in lung function is expected for O_3 exposure. Following exposure, lung function is measured and respiratory epithelial permeability is determined by measuring the lung clearance rate of inhaled aerosolized radiolabeled hydrophilic probe molecules using serial gamma camera imaging. An increase in the clearance rate of the probe represents an increase in epithelial permeability. In addition, the correlation between individual changes in epithelial permeability and lung function resulting from O_3 exposure will be investigated.

Immunological and biochemical studies are playing an increasing role in the clinical research program. Recent studies include investigation of subjects exposed at rest to either filtered air or 0.5 ppm O_3 for 4 hr on 2 consecutive days. The influx of neutrophils, an indicator of acute local inflammatory response, into the upper respiratory tract was examined before and at various times after the exposures by quantification of neutrophils in nasal lavage. When compared to those exposed to clean air, a significant increase in neutrophils occured in subjects exposed to O_3, indicating acute local inflammation. Similar data for subjects exposed to several O_3 concentrations ranging between 0.0 and 0.4 ppm with heavy intermittent exercise are presently undergoing analysis.

The mitogenic response of blood lymphocytes was examined in cells obtained from subjects exposed to several concentrations of O_3 ranging between 0.0 and 0.4 ppm with heavy intermittent exercise. Results suggest a concentration related suppresion of mitogenic response with statistically significant suppresion of response beginning at 0.24 ppm O_3. Because the number of subjects exposed in this study was relatively small, additional exposures to the lower O_3 concentrations are being conducted.

Future studies will focus on the cellular elements and proteins obtained from bronchoalveolar lavage following O_3 exposure. Cell studies will include the accumulation of neutrophils as an indicator of nonspecific inflammation and macrophage lymphocyte immune function as indicator of the status of anti-microbial defense mechanisms. Proteins studied in the lavage fluid will include enzymes capable of causing tissue damage (e.g., collagenase, proteases), plasma proteins as an indication of membrane leakage, and collagen fragments as an indication of tissue damage. The study of lung collagen metabolism using nonradioactive isotopes of proline and hydroxyproline, as well as the presence of collagen metabolites and fragments in the blood and urine of subjects exposed to O_3, is being planned for the near future.

Renewal of investigation into the initial increased responsiveness and subsequent attenuation of responsiveness observed with repeated daily exposures to O_3 is underway. The persistence of increased responsiveness to O_3, methacholine (a bronchoconstrictor) and citric acid (an irritant) following a single O_3 exposure presently is being studied in the same subjects. In addition to information regarding the time course of increased responsiveness to O_3, persistence of increased responsiveness to all three substances may suggest some potential determinants of the increased responsiveness to O_3. Future studies will focus on elucidating the mechanisms for both increased responsiveness and attenuation of responsiveness to O_3.

The responses of several hundred healthy subjects to O_3 have been determined. Data from these subjects are being analyzed to determine if there are demographic, genetic or other factors which are related to intrinsic O_3 responsiveness. In addition, data obtained at different ventilation rates and at different durations of exposure are being analyzed with the goal of establishing more reliable equations relating effective concentration (some combination of O_3 concentration, minute ventilation, and duration of exposure) to decrements in lung function. Preliminary analyses indicate the existance both of a time related threshold and plateau of O_3-induced response, thus creating doubt about the applicability of previous equations.[5]

Studies of Populations with Preexisting Disease. Patients with chronic obstructive lung disease exposed to 0.2 and 0.3 ppm O_3 with light intermittent exercise (VE = 25 l/min) experienced no significant changes in lung function.[6,7] Individuals with allergic rhinitis (hay fever) with no history of asthma like symptoms (eg. wheezing, chest tightness) exhibited decrements in lung function similar to those of healthy subjects when exposed to 0.18 ppm O_3 with heavy intermittent exercise.[8] An O_3-induced increase in airway sensitivity to methacholine (a nonspecific bronchoconstrictor) was observed in these subjects.

Subjects experimentally infected with rhinovirus type 39, a common cold virus, experienced mild upper respiratory infection. With 5 consecutive daily exposures to relatively high O_3 concentrations, these subjects exhibited decrements in lung function similar to those of noninfected subjects exposed under the same conditions. In addition, ozone exposure did not adversely alter the mechanisms which normally act to limit and terminate viral infection of the respiratory tract. Likewise, O_3-induced lung function decrements in individuals with naturally occurring respiratory infections were similar to those of noninfected controls.

Asthma is characterized by airway hyperresponsiveness to a variety of inhaled substances, and it seems likely that asthmatics might be more responsive to O_3 than nonasthmatics. Results from previous studies had indicated that such was not the case for O_3-induced changes in lung volumes and flows. However, changes in airway resistance, a more specific and more sensitive indicator of bronchoconstriction (the hallmark of asthma) were not evaluated in these studies. Thus, methacholine sensitive asthmatic subjects were exposed for 2 hr once to clean air and once to 0.18 ppm O_3; moderate intermittent exercise was performed during exposure.[9] When compared with exposure to clean air, significant increases in airway resistance and decreases in FVC and FEV1 were observed following exposure to 0.18 ppm O_3. While the decrements FVC and FEV1 were comparable to those observed for nonasthmatic subjects under similar exposure conditions, the increase in airway resistance was greater. This indicates that the airways of asthmatics are more responsive to O_3 and is also consistent with the previously stated thesis that two relatively distinct mechanisms are responsible for the O_3-induced changes in these variables.

Presently, responses of asthmatics to intermittent versus continuous exercise during O_3 exposure are being compared to determine the more optimal prodocol. When this is accomplished, asthmatic subjects will be exposed to several low concentrations of O_3 and the relationship between O_3 concentration and changes in airway resistance will be established for each individual subject. The effect of O_3 on airway responsiveness to methacholine will also be evaluated. Ozone concentrations and experimental protocol will be based on the results of the ongoing study. In the future, responses of asthmatics to combined O_3 and sulfuric acid exposure and to sequential exposures to O_3 and sulfur dioxide will be studied.

Nitrogen Dioxide

There are no reports in the current scientific literature that describe decrements in traditional pulmonary function measurements as a result of short term exposures of healthy subjects to relatively low levels of NO_2. Thus, our study of the effects of this pollutant has been confined to the asthmatic population. Methacholine sensitive asthmatics (n = 13) were exposed once to clean air and once to 0.3 ppm NO_2 under cool, dry conditions.[10] Exposures consisted of three cycles of 20 min of rest and 10 min of moderate exercise ($\dot{V}E$ = 40 l/min). Compared to responses in clean air, a small, but statistically significant decrement in FEV1 was observed after the first 10 min of exercise; small and nearly significant decrements in FVC and airway resistance were observed also. Individually, six subjects showed essentially no FEV1 response to NO_2 (< 0.1 liter difference), while seven

subjects demonstrated FEV1 decrements ranging between -0.1 and 0.7 liters. After both the second and third exercise, decrements in FVC, FEV1, and airway resistance were significantly greater for exposure to NO_2 than for clean air.

Presently, asthmatics are being exposed with intermittent moderate exercise to several NO_2 concentrations ranging between 0.0 and 0.6 ppm. Concentration-response relationships (NO_2 concentration vs. lung function responses) will be evaluated for both group and individual data. The effect of NO_2 on airway sensitivity to methacholine also will be evaluated. Nasal lavages are obtained after each exposure and examined for indications of inflammation. In a subset of these asthmatics, urine specimens are obtained 20 hr after exposure and examined for collagen metabolites and fragments. If results from this study and the O_3 studies so indicate, the effects of NO_2 on epithelial barrier function and cellular and molecular constituents harvested from the deep lung with bronchoalveolar lavage will be evaluated.

Carbon Monoxide

Research on CO is conducted by both the Clinical Research Branch and the Neurophysiology Branch of the Neurotoxicology Division. Data have been collected pertaining to the adequacy of various equations for predicting carboxyhemoglobin (COHb) concentrations under various physiological and exposure conditions. The relative sensitivity and importance of the various physiological variables involved in these equations also are being evaluated. These data are presently being analyzed. If warrented, additional studies in this area will be proposed.

A research project also is being designed to study the rate of COHb formation as a function of both CO exposure and exercise level. While much data exist in the literature, particulars are still lacking. Such data are needed to estimate COHb levels resulting from various times and levels of environmental exposure.

The effect of small elevations in COHb concentration resulting from CO exposure on indicators of heart function in patients with ischemic heart disease was evaluated. Patients were exposed once to air (carboxyhemoglobin = 1.5%) and once to 100 ppm CO (COHb = 4.1%) in a double blind, randomized fashion. A standard exercise test was performed after each exposure. Using gamma camera imaging of an injected radioisotope, objective measurements of left ventricular ejection fraction at rest, as well as maximal ejection fraction and left ventricular wall motion abnormalities during exercise were obtained immediately after exposure and during exercise, respectively. More traditional measurements of heart function (eg. time to onset of angina, maximal ST segment depression) also were obtained. Final analyses of these data are being performed. Similar studies at different COHb concentrations have recently begun. Studies planned for the near future will focus on the effects of elevated COHb in patients with diseases other than ischemic disease (e.g., congestive heart failure, intermittent claudication) and in patients with more homogenously defined ischemic disease (eg. abnormal left ventricular ejection fraction response to exercise).

CO dose-effect functions are being studied in humans at exposure levels below and well above threshold. In the past, human studies have been restricted to near-threshold level exposure. The results of these studies have been variable, some indicating effects at a given CO level, others not. By using a wide exposure range to describe a continuum of effects, more reliable estimates of threshold should be attainable. Effects of exposure to CO at low-levels for long times (3 hr) are being compared to exposure to CO at high-levels for short times (5 min). Comparisons will be made between effects at equivalent COHb levels. Results should yield information about possible compensatory mechanisms and possible direct CO toxicity. Variables being studied are: (a) hand-eye coordination in the form of compensatory tracking behavior (b) the spectra of the electroencephalogram, and (c) the ability to discriminate speech in a noisy environment. Also being studied (at low-level 3-hr exposure only) are the effects of COHb on choice reaction time and slow evoked potentials in young and elderly human subjects.

Lead

Because it is well known that children are more sensitive to the neurotoxicity produced by lead than are adults, our approach continues to focus on the consequences of lead exposure early in life. There are three primary questions which our research is designed to answer. First, what are the neurological effects we can expect to see in children with body burdens of lead we would have considered previously to be low? Second, what is the relationship between the body burden of lead and the prevalence or severity of neurological effects, and third, how persistent are these effects?

To answer these questions, we are performing neurobehavioral studies on children identified previously as having elevated blood lead levels and on monkeys with carefully controlled exposure to lead during early life. In both cases some tests which evaluate the electrical activity of the brain and others which evaluate cognitive functions are performed. Presently we have some evidence that dysfunction caused by lead does not appear to demonstrate a threshold. In addition, based upon exploratory data analyses, we are actively investigating the suggestion that very low lead levels may be associated with sensory (auditory and visual) impairments.

Animal Toxicology Research

The Toxicology Branch studies the effects of inhaled O_3 and NO_2 in animals and is developing extrapolation models to relate quantitatively effective pollutant concentrations in animals to man.

Chronic Effects of O_3 and NO_2 in Rats

A wide array of effects are reported as a result of subchronic exposure to O_3 and NO_2, but very few studies documenting chronic effects have been published.[11,12] General conclusions drawn from the entire data base are that these gases can cause chronic lung disease and effects on lung morphology, biochemistry, and anti-microbial host defenses after long-term exposures. However, quantitative evaluation of the few chronic studies is inhibited because most were conducted several years ago using methods less sensitive

than those presently available or because they were designed to address issues not directly related to EPA's needs. Therefore, studies of chronic exposure to O_3 and NO_2 relevant to regulatory issues are being performed.

The major objectives of the chronic study are:

1. To determine the progression of chronic lung disease as a function of alteration of physiology, morphometry, and biochemistry (e.g. collagen/elastin metabolism).

    a. To ascertain the correlations between these experimental parameters.

    b. To determine the progression of lung disease during exposure and post-exposure periods.

    c. To determine whether future studies can be conducted with shorter lengths of exposure and still be predictive of chronic lung disease.

    d. To determine whether there are non-invasive indicators of preclinical chronic lung disease that may be useful in human clinical or epidemiological studies.

2. To determine effects on pulmonary cellular and immunological defenses and to ascertain whether there are systemic immunological effects or correlates measurable by non-invasive methods which may be applicable to potential human clinical or epidemiological studies.

3. To determine effects on lung biochemistry (e.g., anti-oxidant metabolism, xenobiotic metabolism).

4. To determine extrapulmonary effects of chronic exposure.

5. To determine the influence of chronic exposure of O_3 and NO_2 on factors related to pulmonary dosimetry of these gases.

We have chosen to use real-world exposure regimens in our chronic study. Data from the National Aerometric Data Bank (NADB) of the Office of Air Quality Planning and Standards (OAQPS), EPA and from the South Coast Air Quality Management District (SCAQMD) (provided by Dr. Jean Ospital, Southern California Edison) were used to determine frequently occurring daily and weekly profiles of NO_2. Frequently occurring patterns for O_3 levels were examined using NADB data. The exposure patterns chosen represent frequently occurring worst cases for urban summer environments.

The NO_2 exposure will consist of a 0.5 ppm background level for 16 hours, a 6-hr. spike, and a 2 hr down time for service. It will take 1 hr for the concentration to rise from 0.5 ppm to 1.2 ppm and another hr to rise from 1.2 ppm to 1.5 ppm. The exposure will be at 1.5 ppm for 2 hr and take 2 hr to go from 1.5 ppm back to 0.5 ppm. Weekend exposure will be to the background level only. Although these concentrations are higher than ambient, the pattern of exposure mimics ambient conditions. The O_3 exposure will consist of a background level of 0.06 ppm for a period of 13 hr, a 9 hr spike, and a

down time of 2 hr for servicing. The concentration will go from 0.06 ppm to 0.25 ppm and back to 0.06 ppm over a 9 hr period. Integration of the spike portion of the curve shows that the exposure will be equivalent to a square wave that averages 0.19 ppm. Again, there will be only the background exposure level on weekends. These concentrations, as well as the patterns of exposure, occur in the ambient air.

In the study, male rats will be used and will be exposed up to 18 mo. To evaluate the progression of chronic lung disease, several examination periods have been selected (1 wk, 3 wk, 3 mo, 12 mo, 18 mo). To determine whether any observed changes are reversible, post-exposure examination times of 1.5 mo, 6 mo, and 4 mo have been selected for the 3 mo, 12 mo, and 18 mo exposure periods, respectively.

Researchers from several institutions are collaborating with us on the study. They include investigators from Duke University, Northrop Services, Inc., University of North Carolina at Chapel Hill, U. Vermont, Experimental Pathology Laboratories, and Southern California Edison. The study, which is expected to take 4 years to complete, has just begun.

Evaluation of Sensitivity of the Young to O_3 and NO_2

Since the literature[11,12] suggests an age-dependent responsiveness to O_3 and NO_2, studies were performed to compare effects in neonatal and young adult rats. Exposure to the oxidants was for 6 wk from day 1 of birth or 6 wks of age. Ozone exposures were for 12 hr/day, 7 days/wk, to either 0.08, 0.12, or 0.25 ppm. A frequently occurring urban air pattern of NO_2 was used; rats were exposed 22 hr/day, 7 days/wk to a background level of NO_2 on which were superimposed a morning and afternoon 1 hr peak exposure (5 days/wk). Three NO_2 exposure regimens were used: baseline of 0.5 ppm, peak of 1.5 ppm; baseline of 1 ppm, peak of 3 ppm; baseline of 2 ppm, peak of 6 ppm. Lung morphometry and pulmonary physiology were studied. The responsiveness of the neonates to O_3 was not substantially greater than that of adults; morphometric changes in the epithelium of the bronchoalveolar junction were observed,[13,14] and the few changes in lung function occurred at the highest O_3 level.[15] However, the neonates did appear to have a greater responsiveness to NO_2, compared to the young adults, in terms of morphometric changes (J. Crapo, L.-Y. Chang, personal communication). These changes were observed at the lowest NO_2 concentration tested. No major alterations in pulmonary function were found, although there was a significant change in compliance. (M. Stevens, personal communication).

Effects of O_3 and NO_2 on Pulmonary Antiviral Defenses

Research conducted over the last 15 years in our laboratory and in collaboration with Dr. Richard Ehrlich, IIT Research Institute, as well as the work of others,[11,12] has shown that exposures to O_3 and NO_2 cause decreases in lung antibacterial defenses. One of the major targets of such exposures is the alveolar macrophage which participates in antibacterial and antiviral defenses and is an accessory cell in the pulmonary immune system. In spite of this knowledge, the effects of O_3 and NO_2 on antiviral defenses remain virtually unknown. The few studies available present an incomplete and

sometimes conflicting picture. Since viral lung infections are significant from a public health viewpoint, this gap in the data base is of concern. Thus, we and our colleagues at Northrop Services, Inc. have initiated a program to develop a model of influenza virus infection and to develop methods to evaluate pulmonary immunity.

The influenza model utilizes strain A2 and is being developed in both mice and rats. The program will focus on determining sensitive indicators of effects on antiviral immunity that can easily be applied to large scale studies. To this end, investigations focus on mechanisms of antiviral immunity. At present, the kinetics of the three major defenses (interferon, natural killer cells, and cytotoxic T lymphocytes) are being evaluated. Interferon modulation of alveolar macrophage function is also under study. Since the project is in the model development phase, only preliminary studies have been conducted with O_3.

Comparative Potency Studies of NO_2 and NO

A very few studies indicate that NO might be equally as potent as NO_2 in causing systemic immunological effects and alterations in lung morphology. Thus, a pilot study will be conducted this year to compare the effects of NO_2 and NO using the streptococcal infectivity model as an index of the status of lung antibacterial defenses. If NO exhibits effects comparable to NO_2, future studies will include exposure to ambient air patterns of NO, NO_2, and O_3 in sequence/combinations.

Quantitative Extrapolation Modeling

The overall thrust of the extrapolation program was described at the last Annual Meeting of APCA.[16,17] Thus, it will only be described briefly here with a more complete description of newer and future research. The program has 2 major elements - dosimetry and sensitivity. Currently, the dosimetry efforts focus on determining the dose of a pollutant delivered to specific target sites in various regions of the respiratory tract of man and animals. Sensitivity refers to the response of different species to a given dose of O_3 and NO_2. Linkage of these 2 elements will provide the basis for quantitative extrapolation.

The dosimetry element of the program has 2 major foci - experimental and theoretical. The theoretical project is well underway; a mathematical model of the deposition of O_3 and NO_2 in the lower respiratory tract of several animal species and man has been developed.[18-20] Improvements have been made in the formulation of the model for humans and include an evaluation of the influence of exercise on O_3 dosimetry.[21] The next stage in the O_3 dosimetry model for animals which involves incorporation of newer information, is nearing completion. The program is now focusing on refinement of factors that have a large impact on the accuracy of the mathematical model. For example, under cooperative agreements, Dr. James Crapo (Duke Univ.) is obtaining data on lung morphometry of animals and man that have the rigor needed for the model; Dr. Daniel Menzel (Duke Univ.) is determining the reaction kinetics of NO_2 with substances in the liquid lining layers of the respiratory tract since such reactions influence the dose of NO_2 to the underlying tissue; a new project will be initiated to quantitatively characterize the constituents of

mucus in man and animals to more fully incorporate biochemical interactions into the model. To begin experimental validation of the mathematical model, approaches using a non-radioactive isotope of oxygen are being used. Under a cooperative agreement, Dr. John Hayes (Indiana Univ.) has completed development of a method to measure ^{18}O in the tissues of $^{18}O_3$-exposed animals. These methods are now being applied by us to compare experimental deposition of O_3 in surgically accessible regions of the respiratory tract to theoretical deposition.

As mentioned earlier, the mathematical model is for the lower respiratory tract. Thus, dosimetry will be incomplete until the removal of O_3 by the upper respiratory tract can be incorporated into the model. Data are available for guinea pigs and rabbits. Research is underway to obtain nasopharyngeal removal data for the rat, the most extensively used species in O_3 toxicology. The Clinical Research Branch is developing methods to make such measurements in man. For additional validation of the deposition models, total respiratory tract removal of O_3 will be determined in man and animals. Once this research is completed for O_3, similar studies will be conducted with NO_2.

Compared to dosimetry, less is known about species sensitivity to O_3 and, therefore, a program was instituted in this area last year. A concentration-response study of the edemagenic effect of O_3 in 5 species of laboratory animals has been completed.[22] When dosimetry models are available, the dose-response relationship can be quantified. Anticipating differences in species sensitivity to O_3, we are exploring mechanisms that may be responsible for causing these differences. To date, studies have been completed that measure species differences in lung antioxidant levels.[23] Future work will address species differences in antioxidant metabolism and the contribution of such differences to species sensitivity.

A major project has been initiated in collaboration with Northrop Services, Inc. and the Clinical Research Branch to determine species sensitivity of man and animals to the effects of O_3 on alveolar macrophages. The study is designed to determine cellular doseresponse relationships after both *in vitro* and *in vivo* exposure of alveolar macrophages from man and several animal species. Exposures will be to $^{18}O_3$ so that dose can be determined. Mechanisms responsible for the observations also will be explored.

Pulmonary physiology studies are being conducted in unanesthetized animals after exposure to O_3. The animals are also challenged with CO_2 to increase respiratory drive. The goal is to compare responses in several animal species to man.

Summary

As has been described, the Health Effects Research Laboratory has a substantial program underway on automotive criteria pollutants. However, considering the research needs for these pollutants, the program is not all-encompassing. Hopefully, through the combined efforts of all the institutions represented in this session, the major uncertainties in the health data base for automotive criteria pollutants will be resolved.

References

1. W.F. McDonnell, D. Horstman, M.J. Hazucha, E. Seal, E.D. Haak, S.A. Salaam and D. House, "Pulmonary effects of ozone exposure during exercise: dose-response characteristics." J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 54:1345 (1983).

2. W.F. McDonnell, D.H. Horstman, S.A. Salaam and D.E. House, "Reproducibility and distribution of pulmonary responses resulting from ozone exposure." The Physiologist 25:251 (1982).

3. W.F. McDonnell, D.H. Horstman, S.A. Salaam and D.E. House, "Reproducibility of pulmonary responses to ozone exposure." Am. Rev. Respir. Dis. 131:36 (1985).

4. W. Beckett, W. McDonnell, D. Horstman and D. House, "Role of parasympathetic nervous system in the acute lung response to ozone." Fed. Proc. 44:614 (1985).

5. D. Horstman, W.F. McDonnell and S.A. Salaam, "Temporal differences in decrement of forced expiratory volume in one second resulting from exposure to ozone with intermittent exercise." Am. Rev. Respir. Dis. 129:145 (1984).

6. J.J. Solic, M.J. Hazucha and P.A. Bromberg, "Acute effects of 0.2 ppm ozone in patients with chronic obstructive pulmonary disease." Am. Rev. Respir. Dis. 125:664 (1982).

7. H.S. Kehrl, M.J. Hazucha, J.J. Solic and P.A. Bromberg, "Responses of persons with chronic obstructive lung disease following exposures to 0.3 ppm ozone." Am. Rev. Respir. Dis. (in press).

8. W.F. McDonnell, D. Horstman and S.A. Salaam, "Pulmonary responses of allergic rhinitis patients to ozone." Am. Rev. Respir. Dis. 127:160 (1983).

9. D. Horstman, L.J. Roger, W. McDonnell and S. Salaam, "Increased specific airway resistance in asthmatics exercising while exposed to 0.18 ppm ozone." The Physiologist 27:213 (1984).

10. L.J. Roger, D.H. Horstman, W.F. McDonnell, H. Kehrl, E. Seal, R.S. Chapman and E. J. Massaro, "Pulmonary effects in asthmatics exposed to 0.3 ppm nitrogen dioxide during repeated exercise." The Toxicologist 5:270 (1985).

11. E.P.A., Air Quality Criteria for Nitrogen Oxides, Ch. 14, ECAO, Research Triangle Park, EPA-600/8-82-026, Ch. 15 (1982).

12. E.P.A., Air Quality Criteria for Ozone and Other Photochemical Oxidants. Ch. 10, ECAO, Research Triangle Park, External Review Draft-1 (1984).

13. Barry, B.E., F.J. Miller and J.D. Crapo, "Alveolar epithelial injury caused by inhalation of 0.25 ppm of ozone." Adv. Mod. Environ. Toxicol. :299 (1983).

14. Barry, B.F., F.J. Miller and J.D. Crapo, "Effects of inhalation of 0.12 and 0.25 ozone on the proximal alveolar region of rats." Lab Invest. submitted for publication (1985).

15. Raub, J.A., F.J. Miller and J.A. Graham, "Effects of low-level ozone exposure on pulmonary function in adult and neonatal rats." Adv. Mod. Environ. Toxicol. 3:363 (1983).

16. Graham, J.A. and G.E. Hatch, "The integration of species sensitivity and dosimetry data in the extrapolation of ozone and nitrogen dioxide health data from animal to man." APCA, Annual Meeting, Paper No. 84-31.4, (1984).

17. Overton Jr., J.H. and F.J. Miller, "Dosimetry of ozone and nitrogen dioxide in man and animals." APCA, Annual Meeting, Paper No. 84-31.2, (1984).

18. Miller, F.J. A Mathematical Model of Tranport and Removal of Ozone in Mammalian Lungs. Ph.D. Thesis, North Carolina State University, Raleigh, N.C., 1977.

19. Miller, F.J., J.H. Overton Jr., E.T. Myers and J.A. Graham, "Pulmonary dosimetry of nitrogen dioxide in animals" in Air Pollution by Nitrogen Oxides eds. T. Schneider and L. Grant, Elsevier Scientific Publishing Company, Amsterdam, 1982, pp. 377-386.

20. Miller, F.J., D.B. Menzel and D.L. Coffin, "Similarity between man and laboratory animals in regional pulmonary deposition of ozone." Environ. Res. 17:84 (1978).

21. Miller, F.J., J.H. Overton Jr., R.H. Jaskot and D.B. Menzel, "A model of the regional uptake of gaseous pollutants in the lung. I. The sensitivity of the uptake of ozone in the human lung to lower respiratory tract secretions and to exercise." Toxicol. Appl. Pharmacol. in press (1985).

22. Hatch, G.E., R. Slade, A.G. Stead and J.A. Graham, "Species comparison of acute inhalation toxicity of ozone." Fund. Appl. Toxicol. submitted for publication (1985).

23. Slade, R., A.G. Stead, J.A. Graham, and G.E. Hatch, "Comparison of lung antioxidant levels in humans and laboratoary animals." Am. Rev. Resp. Dis. in press (1985).